285

MANIFESTATIONS OF ACUTE AND CHRONIC GRAFT-VERSUS-HOST-DISEASE IN REDUCED-INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION (RISCT) FOR PEDIATRIC CANCER

Baird, K.[1], Fry, T.J.[1], Love, C.[1], Layton, P.[1], Rager, A.[1,2], Cordoza-Salzman, T.[1], Mackall, C.L.[1], Wayne, A.S.[1] [1]National Institutes of Health, National Cancer Institute, Pediatric Branch, Bethesda, MD; [2]Duke University School of Medicine, NC.

Background: Allogeneic stem cell transplantation (alloSCT) plays an important role in the treatment of pediatric malignancies. Unfortunately, its success is limited by toxicities associated with myeloablative preparative regimens. We have piloted a reduced intensity alloSCT (RISCT) approach intended to reduce toxicity and to promote rapid immune recovery and enhanced Graft-Versus-Tumor (GVT) effect. The risk of increased GVT effects may coincide with increased manifestations of GVHD. Although the adult RISCT experience has shown increased GVHD, the pediatric oncology experience is limited. Here we review the unique manifestations of GVHD in pediatric patients undergoing RISCT at our institution.

Methods: We piloted a RISCT regimen in 26 pediatric patients with high-risk hematologic malignancies and sarcomas. Fludarabine-based induction chemotherapy was administered for disease control and targeted CD4 count reduction. Pre-transplant conditioning consisted of cyclophosphamide (1,200 mg/m2/day) and fludarabine (30 mg/m2/day) x 4 days plus melphalan (100 mg/m2 × 1 dose in sarcoma pts). Grafts consisted of G-CSF mobilized unmodified peripheral blood stem cells from 5-6/6 HLA-matched first-degree relatives (median CD34 dose 9.42 × 106/kg; median CD3 dose 387 × 106/kg). Cyclosporine was used for GVHD prophylaxis in both trials, 3 sarcoma patients also received sirolimus.

Results: Twenty-three of 26 recipients developed aGVHD: 18 with grade 1-2, 4 with grade 3, and 1 with grade 4. Twenty-one of 23 patients with >100 days follow-up developed cGVHD. GVHD has been reponsive in most and 6 of 11 surviving remain on treatment. Unique findings in this group include a high incidence of cGVHD (91%), including high rates of bronchiolitis obliterans and eye involvement. We have also seen recurrent GVHD flares associated with cytotoxic chemotherapy given for disease relapse. Interestingly, we have observed rapid immune recovery despite GVHD and the need for systemic therapy.

Conclusions: Despite a significant percentage of recipients experiencing acute and chronic GVHD, the transplant is overall well tolerated. Both trials however were amended to reduce the incidence of GVHD. Sirolimus was added as prophylaxis in sarcoma patients and the stem cell source for those with hematologic malignancies was changed from peripheral blood to bone marrow. With modifications aimed to control GVHD, the RISCT regimen may allow for successful application of this approach in pediatric cancers.

286

ROLE OF RESPIRATORY VIRAL INFECTION IN THE DEVELOPMENT OF ALLO-REACTIVE LUNG DISEASE

Versluys, B.[1], Rossen, J.[2], Bierings, M.[1], Schuurman, R.[2], Ewijk, B. van[3], Boelens, J.J.[1] [1]UMCU/WKZ: Dept. Heamotology/Immunology/BMT, Utrecht, Netherlands; [2]UMCU: Dept. of Virology, Utrecht, Netherlands; [3]UMCU/WKZ: Dept. of Pulmonology, Utrecht, Netherlands.

Background: In lung transplants respiratory viruses (RV) are associated with bronchiolitis obliterans (BO) and rejection. Also in stem cell transplantation (SCT) RV may progress to pneumonia or might trigger immunological mediated effects on lungfunction. We prospectively studied the clinical impact of RV in pediatric SCT patients.

Methods: All patients with respiratory symptoms had a nasopharyngeal aspirate taken, and a PCR on all common RVs was done. The test was repeated weekly. Initial clinical symptoms were noted, as were long term complications. Bronchiolitis obliterans (BO) and Idiopathic pneumonia syndrome (IPS) were reagard as allo-reactive lungdiseases (alloLD) and analyzed as group. A risk factor analyses was performed using logistic regression.

Results: 73 SCTs were done from 40 matched donors and 33 mismatched donors. Median follow up was 17 (5-34) months. Overall survival was 59%. Acute-graft versus host disease (GVHD) > grade I occurred in 25%. Chronic-GVHD occurred in 20%. In 36 pts a RV was identified between day -7 and day +30: rhino-(16), parainfluenza-(3), influenza-(1), adeno- (2) and multiple viruses (11). Three patients with otherwise unexplained typical viral symptoms were considered as RV positive, despite negative PCRs. Initial clinical symptoms were usually mild allthough the virus was not cleared for wks/mths. IPS occurred in 10 pts, at a median of 7 wks post-HCT (range 3-12) of whom 5 died. BO occurred in 7 pts, at 16 weeks post-SCT (range 12-26): Four patients died and 1 is awaiting lung transplantation. In the RV negative group only 1 patient developed a IPS coinciding grade IV aGVHD. In a multivariate analysis only a RV-infection was a risk factor for developing alloLD (p= 0.002, OR 55, range 5-660). Contradictary, aGvHD was associated with a lower incidence of alloLD (OR 0.05, range 0.003-0.80; p=0,034). Perhaps treatment given for aGvHD protected for development of alloLD. The onset of aGvHD was earlier than alloLD.

Conclusion: RV infection early after SCT is associated with increase susceptibility for alloLD. Although the exact role of RV in these complications should be elucidated the fact that aGvHD (and treatment) protected for alloLD suggests allo-reactivity to play a central role. Better isolation methods, close monitoring of pulmonary function and early initiation of immunosuppressive treatment or prolonged immuno-suppressive treatment in RV-positive patients might influence the outcome.

287

REDUCED INTENSITY CONDITIONING ALLOGRAFTING INDUCES THE GENERATION OF ANTIGEN-SPECIFIC REGULATORY T CELLS NECESSARY FOR GRAFT TOLERANCE

Weng, L.[1], Dyson, J.[2], Dazzi, F.[1] [1]Department of Haematology, Imperial College, London, United Kingdom; [2]Department of Immunology, Imperial College, London, United Kingdom.

Reduced intensity conditioning (RIC) regimens have been developed to assist the establishment of host-versus-graft (HvG) tolerance which is required for the subsequent use of donor lymphocyte infusions. Therefore, understanding the mechanisms of HvG tolerance is crucial for maximising the donor lymphocyte mediated graft versus leukaemia (GvL) effect. To address this question we have utilized an animal model whereby sublethally irradiated (400cGy) female recipients were transplanted with bone marrow (BM) cells from syngeneic male donors. Under these conditions, donor cells engraft and HvG tolerance specific for the male HY antigen is established. We observed a selective expansion of T cells with a regulatory phenotype (CD4+CD25+FoxP3+) in the peripheral blood, spleen, and bone marrow of recipient mice. Such expansion was not antigen-dependent nor it depended on the administration of donor hematopoietic cells. In fact, irradiation alone or the irradiation and infusion of female BM cells were sufficient to generate similar levels of Treg expansion. However, when we evaluated the effect of recipient chimeric spleens on HY-specific MLR, an immunosuppressive activity was identified which was only detectable when the mice were transplanted with male but not female BM cells. Such activity was also observed in vivo: the adoptive transfer of chimeric splenocytes prevented in vivo killing of CFSE-labelled male donor cells in female hosts, and enhanced male donor BM engraftment in suboptimally conditioned (200cGy) female recipients. To confirm the role of Treg in this suppressive activity, we depleted chimeric spleen of CD4+ or CD25+ cells and in both cases the suppression was abolished or much reduced. The fundamental role of Tregs in RIC induced tolerance was supported by the fact that administration of anti-CD25 depleting antibodies to conditioned recipients at the time of SCT prevents the induction of Tregs and donor-recipient chimerism. Our data demonstrate that: 1. CD4+CD25+FoxP3+ Tregs are required for the generation of HvG tolerance after RIC allografting and 2. the presence of the antigen during homeostatic expansion induce the generation of antigen-specific Treg.